Short Communication Plant Tissue Culture Letters, **10** (2), 180-183 (1993)

Cell Density Is an Important Factor for Synchronization of the Late Stage of Somatic Embryogenesis at High Frequency

Koichi OSUGA*, Hiroshi KAMADA** and Atsushi KOMAMINE***

**Advanced Technology Research Center, Mitsubishi Heavy Industries, Ltd.*,
8-1 Sachiura, 1-Chome, Kanazawa-Ku Yokohama, 236 Japan
***Gene Experiment Center, Institute of Biological Sciences, University of Tsukuba*,
1-1-1 Tennoudai, Tsukuba-shi, Ibaraki, 305 Japan
****Department of Chemical and Biological Sciences, Faculty of Science, Japan Women's University*,
2-8-1 Mejirodai, Bunkyo-ku, Tokyo, 112 Japan

(Received January 6, 1993)
(Accepted April 21, 1993)

Somatic embryogenesis is useful for the micropropagation of plants and the production of artificial seed. Uniformity of material with a high rate of developmental synchronization are also important features.

Fujimura and Komamine[1] reported high frequency and synchronous embryogenesis in carrot suspension cultures. In their system, however, development from globular to torpedo-shaped embryos was not as well synchronized as earlier stages.

In this paper, we report that density of cells or cell clusters (designated "cell density") is an important factor for synchronization of somatic embryogenesis, especially during development from globular to torpedo-shaped embryos.

Effect of cell density on somatic embryogenesis

Cell cultures used in these experiments were initiated from hypocotyl segments of domestic carrot seedlings (*Daucus carota* L. cv. "Kurodagosun"). Suspension cultures were subcultured every 7 days for 2 to 6 months in a modified Lin & Staba medium containing 5×10^{-7} M 2, 4-D (LS-D medium)[2].

Stock cell suspension cultures (80 m*l*) were grown at 25°C in 300-m*l* flasks on a reciprocal shaker (70 strokes/min. and 5 cm amplitude) in the dark.

Induction of embryo formation was performed according to Fujimura and Komamine[1]. Seven-day-old carrot cell suspensions were sieved through a 50 μm nylon screen and subsequently through one with the 32 μm pores. Cell clusters retained on the 32 μm screen were collected and transferred to a modified Lin & Staba medium without plant growth regulators (LS medium) and grown under conditions similar to stock cultures.

Fig. 1 shows the effect of cell density on somatic embryogenesis. The rate of embryo formation is expressed as the ratio of total formed embryos (*i. e.* sum of globular, heart-shaped, and torpedo-shaped embryos) to the initial number of embryogenic cell clusters (REE, open circle), and the ratio of torpedo-shaped embryos to the initial number of embryogenic cell clusters (RTE, closed circle). Globular and heart-shaped embryos formed at high frequency (80~90%) even at the higher densities of 1×10^3 or 2×10^3 cell clusters/m*l* compared to usual conditions of 5×10^2 cell clusters/m*l*. However, no torpedo-shaped embryos formed at higher densities. Globular and heart-shaped

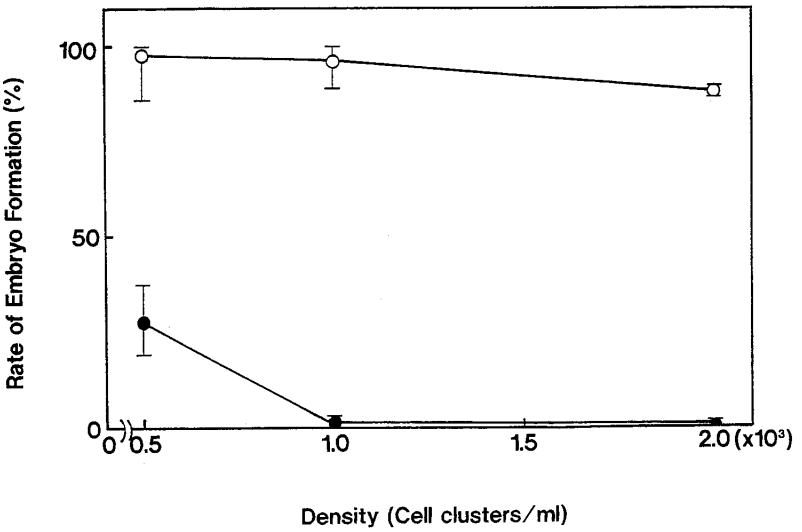

Fig. 1 Effect of cell density on somatic embryogenesis.
Rate of embryo formation is expressed as the ratio of total embryos formed to the initial embryogenic cell clusters (○), and the ratio of the formed torpedo-shaped embryos to the initial embryogenic cell clusters (●). Embryos were counted on the 14th day after transfer to a LS medium. Bars indicate SD ($n=3$).

embryos formed at cell densities of 2×10^3 cell clusters/m*l* cultured over 30 days (in the same medium without transfer), but torpedo-shaped embryos did not form. Furthermore, with cell densities greater than 1×10^3 cell clusters/m*l*, torpedo-shaped embryos did not form. Based on these results, it appears that cell density does not influence development of embryogenic cell clusters to globular or heart-shaped embryos, however, is an important factor thereafter.

Effect of cell density on the late stage of somatic embryogenesis

Cell clusters were cultured at cell densities of 2×10^3 cell clusters/m*l*. After 7 days of culture, globular and heart-shaped embryos were sieved through a 200 μm nylon screen and then through one with 77 μm pores. Globular embryos retained on the 77 μm screen were collected and transferred to LS medium without plant growth regulators. Globular embryos were cultured at various densities below 5×10^2 embryos/m*l*. The number of torpedo-shaped embryos was counted under an inverted microscope after 5 days.

The effect of density on the development of globular embryos to torpedo-shaped embryos is shown in **Fig. 2.** The rate of embryo formation is expressed as the number of torpedo-shaped embryos to the number of initial globular embryos (RTG). When the density of globular embryos was below 150 embryos/m*l*, RTG was high (80~90%). Therefore, density is very important for synchronization of somatic embryogenesis at high frequency, especially during globular to torpedo -shaped embryo differentiation.

The RTG being lowered with the increasing density of globular embryos may be attributed to the following factors: (1) Concentration of conditioning factor or factors which embryos release into the culture medium. Recently A. J. De Jong *et al.*[3] and F. L. Schiavo *et al.*[4] reported that glycosylated acidic endochitinase is released into the culture medium and has an important function in the process in which globular embryos differentiate to torpedo-shaped embryos; (2) Quantity of nutrients which one embryo can take up; (3) Probability of contact among embryos.

In conclusion, the system established here is as follows: embryogenic cell clusters were cultured

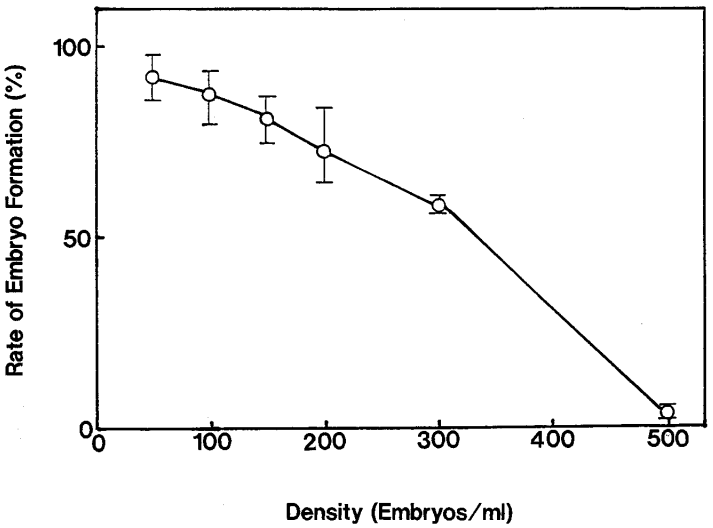

Fig. 2 Effect of embryo density on the development of globular to torpedo-shaped embryos. Rate of embryo formation is expressed as the ratio of torpedo-shaped embryos to initial globular embryos. Globular embryos were cultured for 5 days and the number of torpedo-shaped embryos was counted thereafter. Bars indicate SD ($n=3$).

in LS medium at cell densities of 2×10^3 cell clusters/ml. After 7 days globular embryos which were sieved through a 200 μm nylon screen and retained on a 77 μm screen were cultured in new LS medium at densities below 150 globular embryos/ml. After 5 days globular embryos synchronously differentiated to torpedo-shaped embryos at high frequency (80~90%).

This study was performed through Special Coordination Funds of Science and Technology Agency of the Japanese Government.

References

1) Fujimura, T., A. Komamine, 1979. Plant Physiol., **64**: 162-164.
2) Fujimura, T., A. Komamine, 1975. Plant Sci. Lett., **5**: 359-364.
3) De Jong, A. J., J. Cordewener, F. L. Schiavo, M. Terzi, J. Vandekerckhove,A. V. Kammen, S. C. De Vries, 1992. The Plant Cell, **4**: 425-433.
4) Schiavo, F. L., G. Giuliano, S. C. De Vries, A. Genga, R. Bollini, L. Pitto, F. Cozzani, V. Nunti-Ronchi, M. Terzi, 1990. Mol. Gen. Genet., **223**: 385-393.

《和文要約》

不定胚分化に与える細胞密度の影響

大菅康一*・鎌田　博**・駒嶺　穆***

*三菱重工業㈱基盤技術研究所
**筑波大学生物科学系
***日本女子大学理学部物質生物科学科

ニンジンを対象に不定胚分化に与える細胞密度の影響を定量的に検討した．2,000 個/m*l* の細胞塊密度で Embryogenic な細胞塊から球状胚及び心臓型胚を誘導し，さらに球状胚を 150 個/m*l* 以下の密度で培養すると 5 日後に 80～90% の高頻度で魚雷型胚が得られた．細胞密度は高頻度同調的な不定胚分化に極めて重要なことが明らかになった．